OCCURRENCE AND DETERMINATION OF N-NITROSOPROLINE AND N-NITROSOPYRROLIDINE IN CURED MEAT PRODUCTS

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ABSTRACT

N-Nitrosopyrrolidine (NPYR) has been detected and confirmed at the ppb level in a significant number of fried bacon samples. N-Nitrosoproline (NPRO) has been assumed to be the primary precursor of NPYR, but there are conflicting reports about its precise role. A method was developed for determining NPRO and a survey of 60 cured meat samples was conducted. No NPRO was detected in uncooked, conventionally cured bacon, Canadian bacon, ham, salt pork, pork roll or pastrami. NPRO was detected and confirmed in 1 of 7 dry cured bacon samples (106 ppb), 8 of 12 dry cured ham samples (18–604 ppb), and 5 of 6 samples of pork side meat (86–411 ppb). The results suggest the NPRO may not be the main precursor of NPYR in bacon, but may have a role in nitrosamine formation in dry-cured products.

INTRODUCTION

THE CONSISTENT occurrence of N-nitrosopyrrolidine (NPYR) in fried bacon has led to an intensive search for both the precursors and mechanism that could account for the formation of this nitrosamine. The precursors of NPYR have been associated with bacon adipose tissue (Fiddler et al., 1974; Mottram et al., 1977). The fact that N-nitroso-3-hydroxypyrrolidine also has been found occasionally in bacon at the low ppb level (Janzowski et al., 1978; Lee et al., 1978; Sen et al., 1976a) suggests that collagen may be the precursor of the two nitrosamines, since collagen consists primarily (ca 46%) of proline, hydroxyproline and glycine. Free proline is present in pork bellies at a concentration of approximately 20 ppm (Lakritz et al., 1976). This amount of proline is sufficient to account for the NPYR normally detected in bacon at the ppb level (Hwang and Rosen, 1976). In a recent report we proposed that free proline could react with nitrite, which is present in the cure solution, to form N-nitrosoproline (NPRO), and then decarboxylate to yield NPYR under conditions normally recommended for frying (Kushnir et al., 1975). Sen et al. (1976b), however, report that preformed NPRO is not the primary precursor of NPYR, as shown by the fact that ascorbyl palmitate, when added to raw bacon, inhibits the formation of NPYR. Hansen et al. (1977) also claim that NPRO is not the main precursor of NPYR in fried bacon. Nakamura et al. (1976) and more recently Coleman (1978) claim that NPYR is formed from pyrrolidine reacting with nitrite and not by the decomposition of NPRO. Bills et al. (1973) demonstrated that putrescine and spermidine also can form NPYR under model system conditions simulating bacon frying. The reason for this controversy is the lack of a sensitive and reliable method for the detection of NPRO. Therefore, because of the conflicting reports describing the precise role of NPRO in bacon, we are reporting in this paper a survey of 60 cured meat samples examined for the presence of NPRO by a new method that is more sensitive than the procedure we previously reported (Kushnir et al., 1975).

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EXPERIMENTAL

Materials

NPYR, NPRO and N-nitrosopipecolic acid (NPIC) were synthesized and purified as reported previously (Pensabene et al., 1972). Acetonitrile, dioxane, ethyl acetate and hexane were "Distilled in Glass" solvents purchased from Burdick & Jackson Laboratories and used without further purification; whereas, diethyl ether and methanol were distilled prior to use. Diazomethane was prepared from Aldrich N-methyl-N-nitroso-p-toluenesulfonamide as directed. The Dowex 21K anion exchange resin was pretreated by successive washings with 50 ml water, 50 ml of 1N NaCl and 250 ml water.

Determination of NPRO

Cured meat samples were ground twice in a mixer equipped with a 1/8 in. plate and thoroughly mixed prior to analysis. A 50-g sample of the ground meat was spiked with 1 ml of a methanol solution containing either 2.5 μg/ml or 10 μg/ml NPIC, mixed well and allowed to stand for 30 min to assure complete penetration of the spike. The fat was removed from the sample by refluxing it with 150 ml of hexane for 15 min. The solvent was decanted from the sample and 150 ml of cold ether was added to the meat residue in the flask to remove any remaining fat. After standing 15 min the ether was decanted and the remaining ether was evaporated from the sample in a stream of nitrogen. The defatted sample was homogenized for 2 min with 150 ml of a mixed solvent system comprised of 1N HCl, acetonitrile and dioxane (2:1:1). The sample was centrifuged for 15 min at 5000 rpm in a refrigerated centrifuge at 2–6°C. The supernatant (pH < 2) was filtered through glass wool into a 500 ml separatory funnel. The residue was rehomogenized as described above to insure complete extraction. The combined filtrates were extracted 3 times with 125 ml of ethyl acetate, and the combined extracts were transferred to a round bottom flask and dried on a vacuum rotary evaporator in a 35–40°C water bath. The residue in the flask was redissolved in 10 ml of a methanol-water (1:1) solution and transferred to a 20 × 300 mm chromatographic column packed with 10g of 50–100 mesh Dowex 21K anion exchange resin. Artifacts were removed from the column with 75 ml of water. The sample was then eluted with 175 ml of 0.5N HCl solution. The acidic eluent was extracted 3 times in a separatory funnel with 100 ml of ethyl acetate, the combined extracts were passed thru anhydrous Na_2SO_4 held in a coarse fritted glass funnel, then dried on a rotary evaporator. The residue in the flask was dissolved in 1 ml of absolute methanol and transferred to a 16 × 145 mm test tube. Three ml of an ether solution containing ca. 1% diazomethane was added, and the tube was heated and shaken for 20 min at 33°C as described by Wolfram et al. (1977). The sample was transferred from the tube to a 4 ml concentrator tube with ether and concentrated to 1 ml prior to gas-liquid chromatography-Thermal Energy Analyzer (GLC-TEA) detection and quantitation.

Determination of volatile nitrosamines

A representative portion of each cured meat sample was fried for 4–6 min in a preheated Presto Teflon-coated electric frying pan calibrated at a temperature of 177°C (350°F). The edible portion and all rendered drippings were retained for subsequent nitrosamine analysis. The samples were analyzed for nitrosamines by procedures described previously (Fiddler et al., 1978).

Detection and quantitation

Volatile nitrosamines were determined directly (Fiddler et al., 1978), and NPRO and NPIC, the internal standard, were detected as their volatile methyl esters by GLC-TEA. The TEA conditions were similar to those described previously (Fiddler et al., 1978). The Varian Aerograph Model 2700 gas chromatograph was equipped with a 9 ft × 1/8 in. stainless steel column packed with 15% Carbowax 20M-TPA on 60–80 mesh Gas Chrom P, operated isothermally at 215°C. Meat samples were spiked with either 50 ppb or 200 ppb

NPIC, and the average recoveries of NPIC were 74% and 77%, respectively. The minimum detectable level of NPRO was 10 ppb. The nitrosamine results reported are the average of two determinations per sample.

GC-Mass spectral confirmation

NPYR and NPRO methyl ester were confirmed by use of the following system: a Varian Aerograph Model 2700 gas chromatograph was interfaced with a Varian Mat 311A mass spectrometer. The gas chromatograph was equipped with a 6 ft × 1/8 in. glass column packed with 15% Carbowax 20M-TPA on 60–80 mesh Gas Chrom P, the helium flow rate was 15 ml/min, and the temperatures used were: detector, 200°C; injection port, 200°C; GLC-mass spectrometer interface, 180°C; and column programmed from 150° to 190°C at 4°/min for NPYR and operated isothermally at 180°C for NPRO methyl ester. The mass spectrometer was operated in the peak matching mode adjusted to a resolution of 1 in 10,000 or 12,000. The mass spectra were measured at an ionizing voltage of 70 ev and an ion source temperature of 150°C. The mass-to-charge ratios (m/e) of 100.06366 for NPYR and 158.069136 for NPRO methyl ester were determined by use of the m/e 99.99361 and m/e 154.992011 perfluorokerosene reference peaks, respectively, by measuring the difference in m/e.

RESULTS & DISCUSSION

THE METHOD for the detection of NPRO described in this paper was applicable, without modification, to all of the various cured meat products analyzed. A typical chromatogram is shown in Figure 1. The nitrosamines can also be quantitated on a gas chromatograph equipped with an alkali flame ionization detector (AFID) and a three-way valve, which is used to direct the solvent from the detector. The use of this valve results in a more sensitive and reproducible signal than would normally be obtained without it. However, even with this valve the AFID chromatogram is not as clean as that obtained by the TEA detector, but it still can be used for quantitation.

Sixty cured meat samples were analyzed by our method for the presence of NPRO, with the results shown in Table 1. Contrary to our earlier report (Kushnir et al., 1975), no NPRO was found in any of the 17 raw bacon samples tested, despite the fact that all the sample had confirmable levels of NPYR in the fried product (5–63 ppb). Our results support the conclusions reached by other investigators (Hansen et al., 1977; Sen et al., 1976b) that free NPRO is not present in sufficient quantities in bacon to account for the high levels of NPYR found in fried bacon.

No NPRO or NPYR was detected in Canadian bacon, conventionally cured ham, or other cured meat products such as salt pork, pork roll, pastrami or corned beef. NPRO (106 ppb) was detected in 1 of 7 samples of dry cured bacon even though all of the bacons had higher NPYR values than conventionally cured bacon after frying (34–89 ppb). The nitrite Safety Council has also reported high levels of NPYR in dry cured bacon (Food Chemical News, 1978). NPRO (18–604 ppb) was also detected and confirmed in 8 of 12 dry cured ham samples and the corresponding fried products all had confirmable levels of NPYR (3–54 ppb); however, high levels of NPYR were not always correlated with high levels of NPRO. Occasionally, N-nitrosodimethylamine (NDMA, 1–5 ppb) was detected in fried dry cured bacon and ham. The presence of NPYR in these dry cured products may result from the long exposure of the nitrite containing curing salts with meat components that serve as the nitrosamine precursor.

In 5 of 6 samples of pork side meat obtained from three producers, NPRO (86–411 ppb) was found. Pork side meat is a dry cured, pepper-coated product similar to back fat, used for flavoring and cooking purposes. This commercial product, although a specialty product not in common usage, contained high levels not only of NPYR (19–149 ppb), but also NDMA (12–51 ppb) and N-nitrosopiperidine (NPIP, 5–35 ppb) after frying. Previous research by Gough and Goodhead (1975) and Sen et al. (1973) showed that cure pre-mixes containing spices, such as black pepper and paprika, can form NDMA, NPIP and NPYR. These spices could then serve as a source of nitrosatable amine when added to cured meat products such as pork side meat. Eisenbrand et al. (1976) reported the occurrence of NDMA, NPIP and NPYR in a peppered ham product. This evidence suggests that spice-coated cured meats have the potential for forming nitrosamines in high concentrations. Therefore, these products should be surveyed more extensively in the future for the presence of volatile and non-volatile nitrosamines.

Our results indicate that NPYR is formed in cured meat products which do not contain detectable levels of NPRO

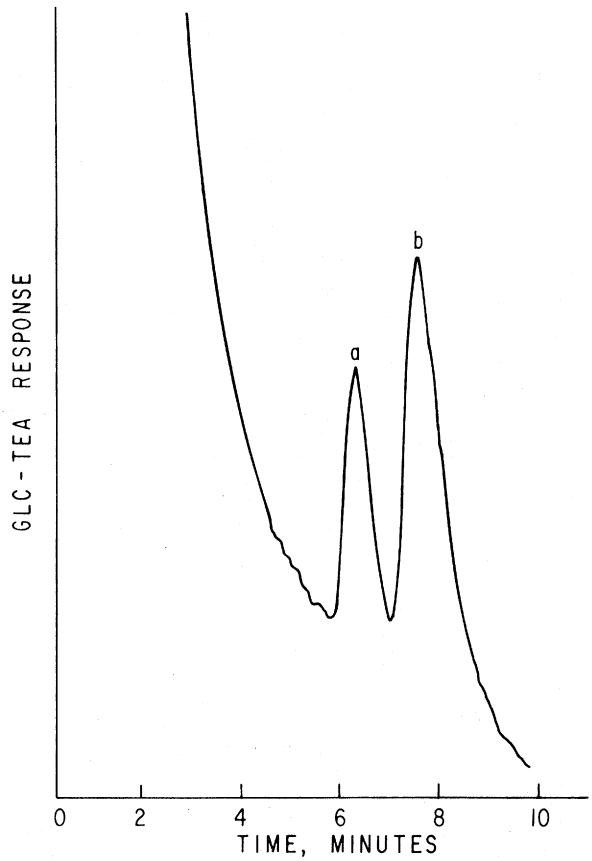

Fig. 1—GLC-TEA chromatogram of an extract from pork side meat with 50 ppb N-nitrosopipecolic acid added: (a) N-nitrosopipecolic acid methyl ester (80% recovery as free acid); (b) N-nitrosoproline methyl ester (63 ppb as free acid).

Table 1—Nitrosamines in cured meat products

Sample type	NPRO[a,b,c,d] No. positive/total	NPRO[a,b,c,d] µg/kg	NPYR[a,b,e] µg/kg
Bacon	0/17	N.D.[f]	5–63
Dry cured bacon	1/7	106	39–89
Canadian bacon	0/5	N.D.	N.D.
Ham	0/5	N.D.	N.D.
Dry cured ham	8/12	18–604	3–54
Pork side meat	5/6	86–411	19–149 (12–51 NDMA; 5–31 NPIP)
Other cured meat products	0/8	N.D.	N.D.

[a] Corrected for recovery of internal NA standards
[b] Confirmed by M.S.
[c] Minimum detectability 10 µg/kg
[d] Uncooked samples
[e] After frying
[f] N.D.—none detected

by our method. It is likely therefore, that NPRO is not the main precursor for NPYR in bacon. However, NPRO may play a significant role in NPYR formation in dry cured meat products based on our results with dry cured products. Nitroso derivatives of amino acids found in food products still require further investigation to determine their role in nitrosamine formation.

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Note: Precaution should be exercised in the handling of nitrosamines since they are potential carcinogens.

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